LPBF
SAVE OUR LAKE
SAVE OUR COAST
LAKE PONTCHARTRAIN BASIN FOUNDATION

May 27, 2020

Danny Wiegand, P.E.
EPA Gulf of Mexico Program
U.S. Environmental Protection Agency
2510 14th Street
Gulfport, MS 39501

RE: A NOVEL & INTEGRATED WASTEWATER-CENTRIC APPROACH TO WATER QUALITY IMPROVEMENT AND COMMUNITY ENGAGEMENT, Quality Assurance Project Plan, MX-00D68218-0

Dear Mr. Wiegand,

The Lake Pontchartrain Basin Foundation (LPBF) is pleased to submit its Quality Assurance Project Plan (QAPP) for the above referenced project. In light of the business interruption caused by COVID-19, we wanted to transmit this electronically signed document to you directly.

I will be able to collect all of the hard signatures on Monday, June 1 and mail to you at that time, we will be sending a hardcopy in the mail to your office at EPA. However, if there is anything in the document that requires further clarification, please do not hesitate to let us know.

Please contact Ridgely Myers or myself if you have any questions.

Sincerely,

Brady K. Skaggs, Ph.D.
Water Quality Program Director
Lake Pontchartrain Basin Foundation

cc: Ridgely Myers, Kristi Trail, Tanya Vidal

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A NOVEL & INTEGRATED WASTEWATER-CENTRIC APPROACH TO WATER QUALITY IMPROVEMENT AND COMMUNITY OUTREACH, QAPP

MX-00D68218-0

Brady Skaggs, Ph.D.
Principal Investigator
Water Quality Program Director

Lake Pontchartrain Basin Foundation
Corporate Office
3501 N. Causeway Blvd.
Metairie, LA 70002

Submitted May 27, 2020

Project Approval (A1)

EPA Project Officer: Danny Wiegand

Signature DANNY WIEGAND Digitally signed by DANNY WIEGAND Date: 2020.06.26 10:30:29 -05'00' Date _____

LPBF Executive Director/ QA Senior Manager: Kristi Trail, P.E.

Signature _____ Date 5/27/2020

LPBF Water Quality Program Director: Brady Skaggs, Ph.D.

Signature _____ Date 5/27/2020

QA Technical Manager: Ridgely Myers, P.E.

Signature _____ Date 5/27/2020

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Figures and Tables:

Distribution List (A3)

U.S. Environmental Protection Agency
2510 14th Street
Gulfport, MS 39501

- Danny Wiegand, EPA Project Officer, Region 4

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- Kristi Trail, P.E., LPBF Executive Director
- Ridgely Myers, P.E., QA Technical Manager
- Brady Skaggs, Ph.D., Water Quality Program Director
- Tanya Vidal, Business/Program & Grants Manager
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- John O'Donnell, Water Quality Specialist
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- Kevin Caillouet, Ph.D., Director & Research Entomologist
- Nick DeLisi, Entomologist & Lab Manager

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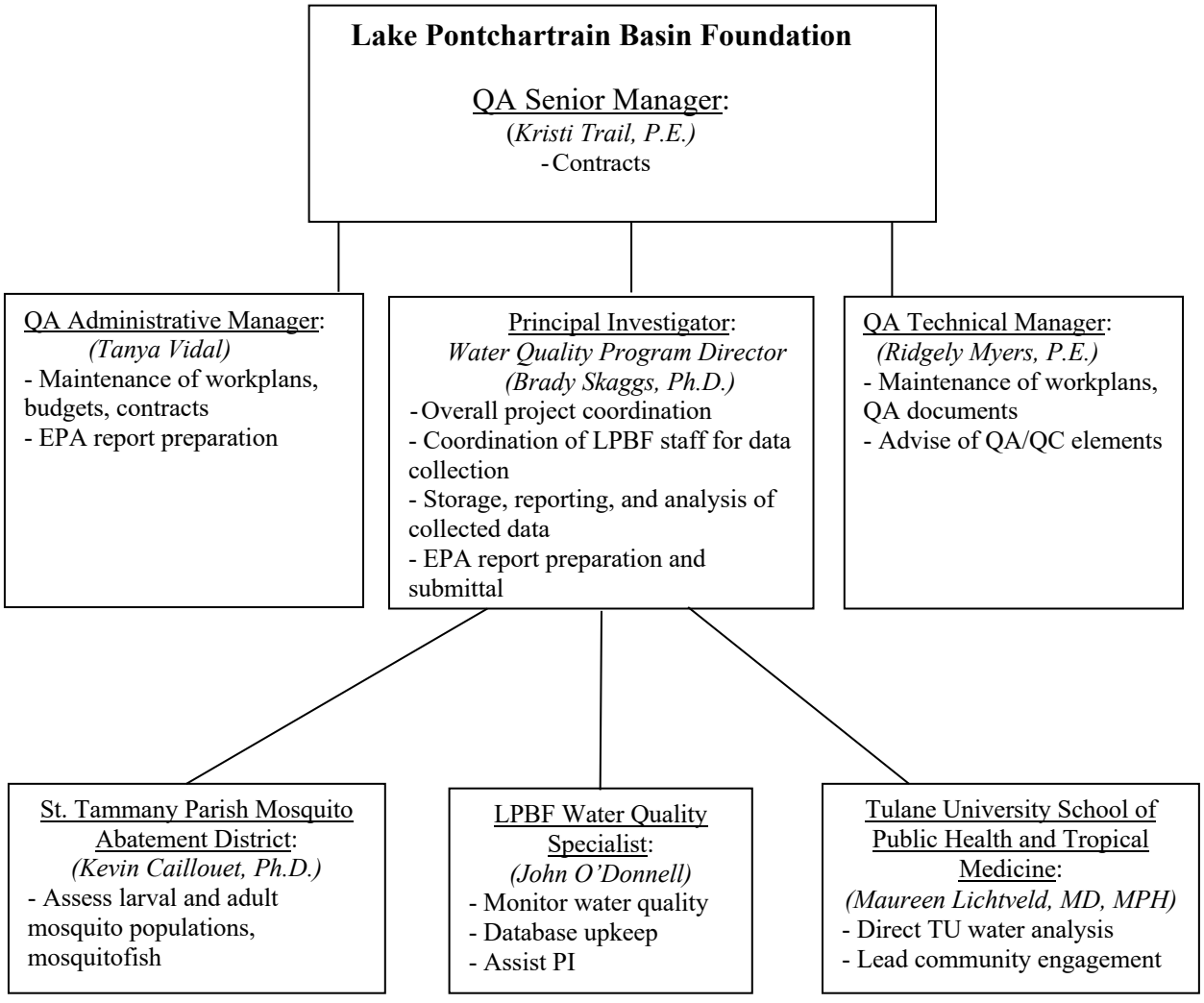
1440 Canal Street
New Orleans, LA 70112

- Maureen Lichtveld, MD, MPH, Professor and Chair at Tulane University
- Dr. Tiong G. Aw, Ph.D., Assistant Professor, Tulane University
- Dr. Samendra Sherchan, Ph.D., Assistant Professor, Tulane University
- Dr. Stephen Murphy, Ph.D., Assistant Professor, Tulane University

Project/Task Organization (A4)

The Lake Pontchartrain Basin Foundation (LPBF) will assess the performance of installed aerated treatment units and provide data on the performance of these units. The project will assess water quality, pollutants, and pathogens; quantify populations of mosquitos that are vectors of human diseases; and assess wastewater conditions' impact on mosquitos and their predation. The project will provide new information about the presence of viral indicators and organic compounds downstream of wastewater treatment units. LPBF will be responsible for water quality sampling, data storage, analysis, quality assurance, and overall sampling logistics of the project. An organizational chart is presented in *Figure 1*.

Figure 1: Organizational Chart



Problem Definition/Background (A5)

This project addresses a pervasive water quality problem in rural and developing areas of Louisiana by bringing an interdisciplinary approach to one watershed in southeast Louisiana. The root of the surface water problem is a permissive state policy allowing high density developments of single family residences equipped with Aerated Treatment Units (ATUs) for wastewater management. This situation is compounded by impervious clay soils and annual average rainfall of approximately 60 inches per year statewide.

Home ATU systems have a high failure rate nationally, and LPBF has observed neighborhood failure rates exceeding the national average greater than 60% of the installed systems. Louisiana's topography and geological conditions, when coupled with failed systems, can lead to stagnating, untreated wastewater in drainage ditches in homeowners' front yards. Fecal coliform, as an enteric pathogen indicator, can be measured to quantify the presence of fecal waste material from warm-blooded animals and sewage pollution. However, the scientific literature casts doubt that fecal coliform may be a sufficient indicator to warn of the presence of disease-causing enteric pathogens for all environmental conditions. This public health problem merits examination in Louisiana.

Effluent produced by improperly maintained ATUs discharging into drainage ditches may enhance mosquito production, and therefore, the possibility of increased pathogen transmission by mosquitoes. Frequently malfunctioning residential ATUs and open, poorly-draining ditches together exacerbate the production of West Nile Virus (WNV) vector mosquitoes.

The proposed project area, Ponchitolawa Creek Watershed, drains approximately 9,441 acres in St. Tammany Parish and is a tributary of the Lower Tchefuncte River, which flows into Lake Pontchartrain (*Figure 1*). As a direct tributary of the Tchefuncte River, Ponchitolawa Creek is designated a Scenic River by the Louisiana Legislature and the Louisiana Department of Wildlife and Fisheries (LDWF) and is considered an Outstanding Natural Resource Water (ONRW) by the Louisiana Department of Environmental Quality (LDEQ).

On the Lower Tchefuncte River, low levels of dissolved oxygen were identified by the Louisiana Department of Environmental Quality as likely related to numerous individual commercial package plants and individual residential treatment units discharging within the watershed. Stream segments have been on the impaired waters list since 2012, for low dissolved oxygen.

The project watershed is within St. Tammany Parish, which has a watershed management plan developed by the Louisiana Coastal Protection and Restoration Authority that identified:

- 20 subdivisions are not connected to community or centralized wastewater treatment systems;
- More than 1,686 residences that have septic tanks or Aerated Treatment Units (ATUs),
- 800[1] homes (2,400 persons) are in Low to Moderate Income (LMI) neighborhoods.

Past LPBF research has shown a high percentage of home wastewater systems to be malfunctioning. In addition, recent LDEQ monthly water quality monitoring on Ponchitolawa Creek (2013-2016) has shown sites at I-12 and at Hwy 59 do not meet new *Use and Attainability*

[1] 800 high-need LMI residences * 3 persons per home = 2,400 LMI persons impacted

Analysis (UAA) Dissolved Oxygen (DO) criteria applicable to this area. LDEQ ambient water quality monitoring data will serve as the baseline for this project.

As shown in *Figure 2* the LDEQ has an Ambient Water Quality Monitoring (WQM) site downstream of the confluence of the Ponchitolawa Creek with the Tchefuncte River. The site is sampled monthly for 29 parameters; all data is available monthly on LDEQ's website. St. Tammany Parish contracted with LPBF to conduct a three-year program of *in situ* stream monitoring for dissolved oxygen and various other regulatory and habitat parameters. This program was concluded in July of 2016, but results are utilized for targeting pollution and source tracking. Projects of this nature may be utilized during this study in Ponchitolawa Creek watershed. There are six such sites in the Ponchitolawa Creek watershed.

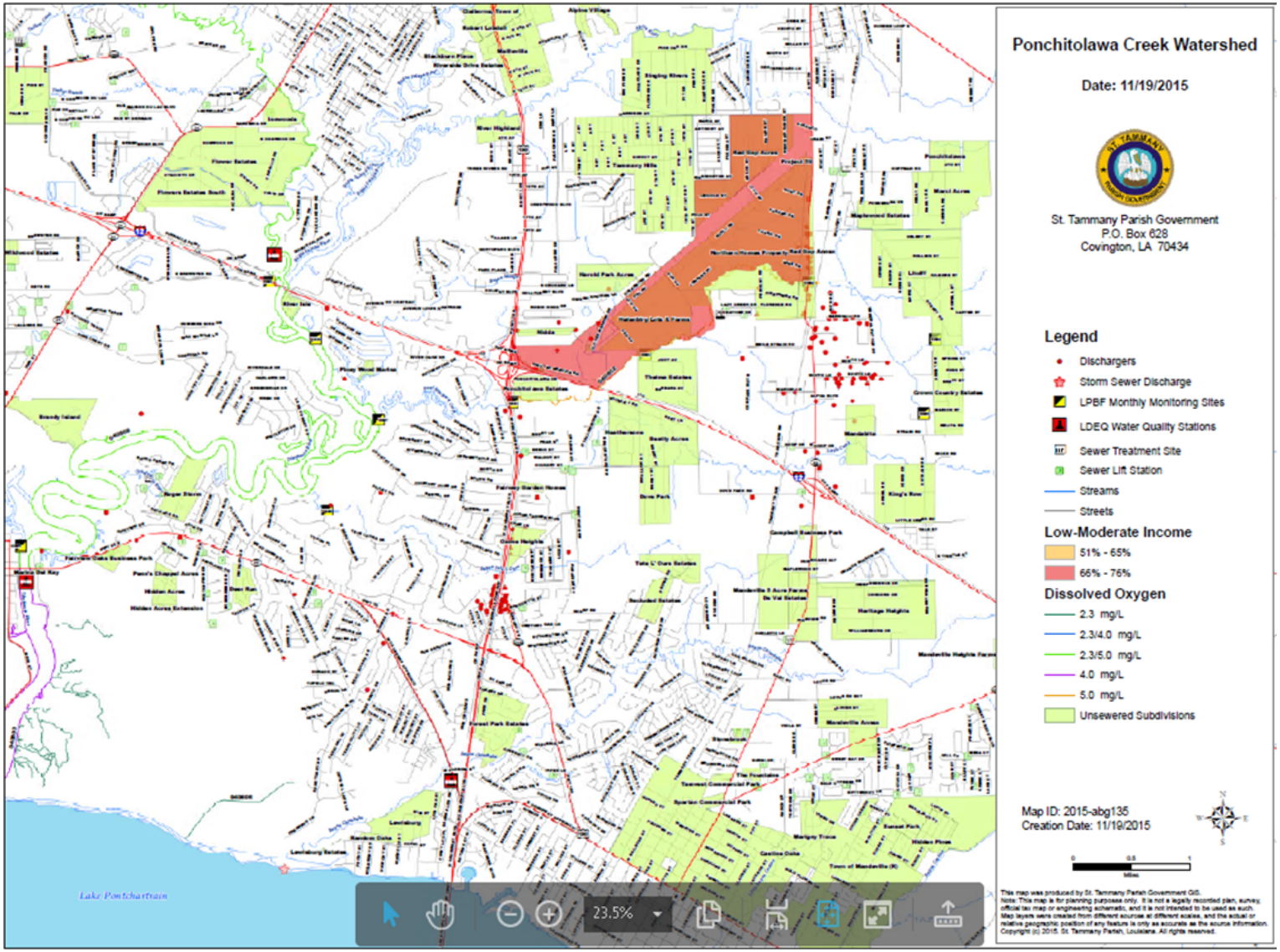
These data will provide continued corroborating information that the watershed continues to benefit from better homeowner treatment system management due to grant funding of the proposed homeowner treatment plant inspection program. Home wastewater systems are considered Nonpoint Sources (NPS) and are therefore not under the regulatory authority of the EPA or LDEQ. These sources are predominantly underperforming wastewater treatment plants or individual residential treatment plants. Residential units are allowed in areas of St. Tammany Parish where there are no central treatment plants within a specified distance. While the Parish is implementing regionalization, decentralized management of residential units seems to be a viable alternative.

Poor water quality can also have lasting impacts on aquatic species within a basin. Within the 20 unsewered subdivisions in the Ponchitolawa Creek Watershed, there are estimated to be more than 1400+ residences that have septic tanks or Aerated Treatment Units (ATUs), about half of which (800 residences) are an underserved, high-need community as designated by LMI statistics in 2010 census. General meetings coordinated by the Parish Councilman, the Parish and its partner, Lake Pontchartrain Basin Foundation (LPBF), will be open to all of the 1400+ residences (potentially 5,000 persons) and local businesses in the watershed. Further, at approximately 100 inspections per month, it is estimated that 400[2] individual residences will receive one-on-one tutorials and brochures of proper operation and maintenance of their individual treatment units

LPBF will inspect these wastewater ATUs, and the units will be repaired or replaced if needed. The water quality monitoring portion of this project targets monitoring of key outfalls from the Ponchitolawa Creek watershed and the receiving waterbodies (upstream and downstream of the influence from the subdivision). This will help quantify the in-stream improvements to water quality as a result of addressing NPS sources, or home wastewater systems

[2] Over four hundred (400) homes will have an initial inspection. Assuming EPA's initial failure rate of 50%, 200 homes will need a second inspection, and 100 homes will require a third follow-up inspection. Therefore, total number of inspections are estimated at 700 total inspections.

Figure 2: Ponchitolawa Creek Watershed with LMI, LDEQ Ambient WQMS and STP Monitoring Sites



Project/Task Description (A6)

Project and Objectives

The objectives of this project are:

1) a reconnaissance and intervention methodology resulting in at least 5% improvement in fecal coliform and dissolved oxygen in Ponchitolawa Creek, and
2) a communication methodology (and materials) that could be replicated across Louisiana and other states.

Through conducting this project, LPBF proposes to substantially decrease waste material entering ditches in targeted neighborhoods in Ponchitolawa Creek. In reducing these non-point source inputs, LPBF seeks to gain at least a 5% improvement in DO and fecal coliform in Ponchitolawa Creek (much greater improvements are expected), which will ultimately improve low DO conditions in the Lower Tchefuncte. The project will also produce an innovative reconnaissance matrix to target neighborhoods in need of assistance, educational materials, and a long-term strategy plan for municipalities, stakeholders, and homeowners.

The project activities are broken into three phases:

1) *Assessment of ATU-Utilizing Neighborhoods in Ponchitolawa Creek Watershed:* A reconnaissance phase to be conducted by LPBF and project partners Tulane University (TU) and St. Tammany Mosquito Abatement District (STPMAD) to create a decision tool/matrix. This phase will involve the following tasks:
    - Assess 20 ATU-utilizing neighborhoods in Ponchitolawa Creek by visual inspection of ditches holding standing sewer water to evaluate the population demographics, proximity of the ditches to public areas and vulnerable populations, and historic mosquito data from STPMAD. This will result in the choice of ten (10) neighborhoods for further analysis.
    - Perform "Phase I" analysis of ten (10) ATU-utilizing subdivisions for enteric pathogen indicators (fecal coliform), adult and larvae mosquito populations, and a suite of pathogens through the Luminex x-Tag system. The Luminex system will detect the presence of gastrointestinal and other waterborne diseases, including: *Campylobacter*, *Clostridium difficile* (Toxin A/B), Enterotoxigenic *E. coli* (LT/ST), Siga-like toxin producing *E. coli* (STEC), *Salmonella*, *Shigella*, *Vibrio cholera*, *Giardia*, Norovirus GI/GII, Rotavirus, *E. coli* O157H7, *Yesinia Entercolitica*, Adenovirus 40/41, *Cryptosporidium*, *Entamoeba histolytica*. Each site will be sampled ten (10) times, roughly monthly, over the course of a year. Water Quality measurements will be collected for pH, DO, conductivity, temperature, and turbidity.
    - The presence of immature (larvae and pupae) mosquitoes and fish predators and water quality parameters will be monitored for correlation with condition of individual residence's sewage treatment system via multiple linear regression. This data will be collected for one month at approximately 400 individual residences.
    - Statistically analyze and map data from above tasks to determine the neighborhoods to be targeted for Phase II.
2) *Intervention Program in an ATU-Utilizing Neighborhood:* An intervention phase to be conducted by LPBF and STPMAD to inspect home ATUs and assist/educate homeowners while quantifying improvements to ditches. This phase will involve the following tasks:
    - Notify the targeted neighborhood(s) of the inspection program through the use of door-hangers and neighborhood signage.

- Perform home ATU system inspections (approximately 100 ATU per month, for a total of 800-900 within the project), including assessment of the condition of the system and homeowner education.
- Document water quality improvements through the intervention by monitoring up to 10 sites monthly within the targeted neighborhood for water quality parameters (e.g.: fecal coliform, DO) to assess changes due to the intervention.
- STPMAD will further assess mosquito populations. In the selected intervention neighborhood(s) and in a similar neighborhood not scheduled for remediation (the control), STPMAD will sample adult mosquitoes prior to and after the intervention to determine the effect of the intervention on the production of adult mosquitoes. Emergence traps, designed to sample mosquitoes that reproduce in an aquatic habitat, and adult mosquito traps, which sample free-flying mosquito populations, will be used in this pre/post intervention study. In addition, water quality parameters and presence/absence of mosquito predators will be measured and noted.

3) *Community Education and Outreach:* LPBF will develop strategies and materials to deliver outreach and education programming to nearby communities. Key messages will include explaining the project and its goals, the science behind water quality, and the roles of individuals in risk-reduction and water quality. A primary goal of the education and outreach component is to raise awareness of individual responsibilities, and to encourage support of investment in public infrastructure, health, and water quality.

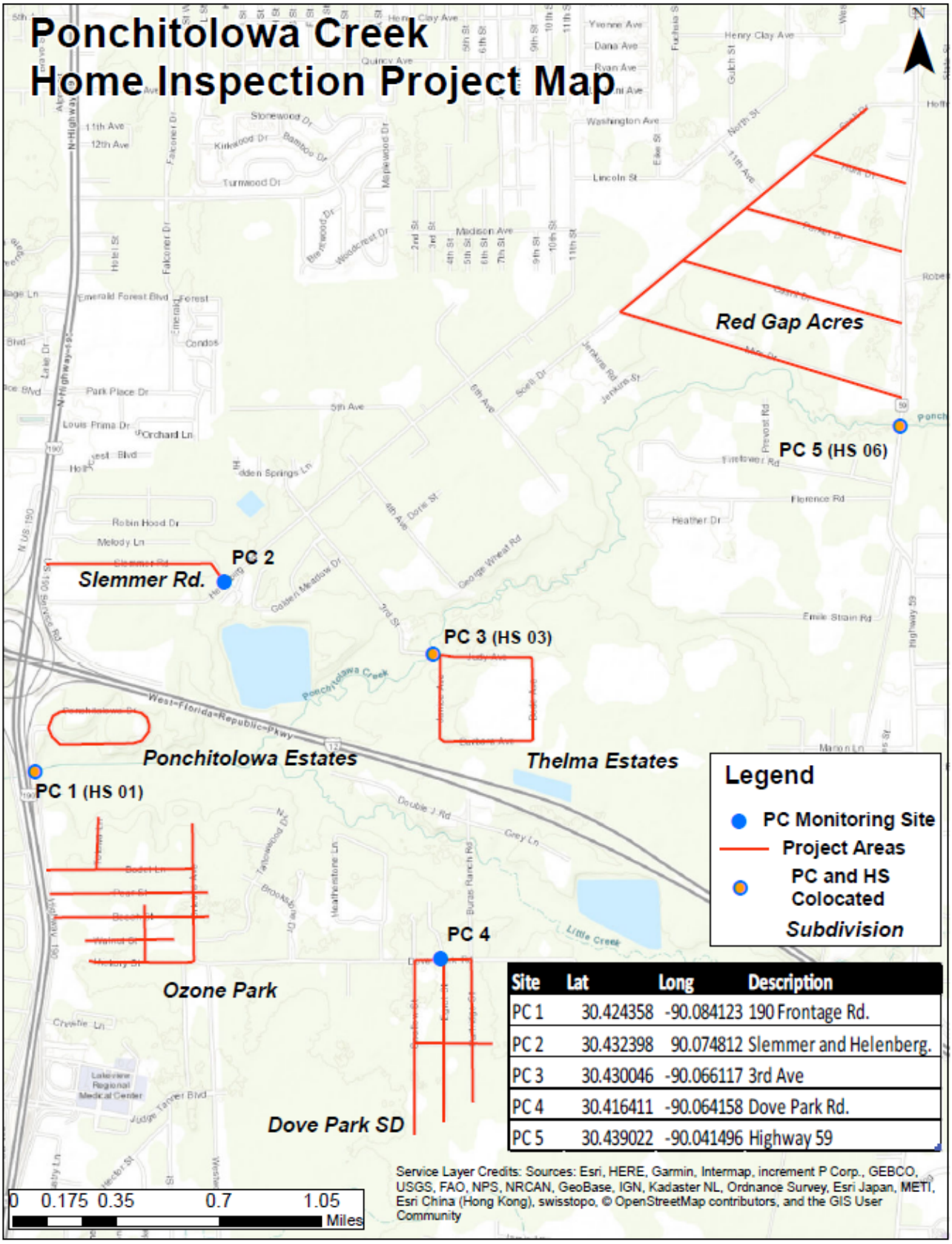
Sampling Locations and Methodology

This QAPP, **A Novel & Integrated Wastewater-Centric Approach To Water Quality Improvement and Community Outreach,** addresses QA/QC requirements for this project. Three efforts are outlined in this document:

- (LPBF) Generation of field-collected data by *in situ* measurement of the physiochemical parameters, and the collection of sample aliquots for further analysis by project partners;
- (STPMAD) Generation of field-collected data by in situ measurement of mosquitos and mosquito fish populations; and
- (TU) Bacteriological analysis of pathogen indicators (*E. coli* and fecal coliform) and pathogen indicators using novel methods for parameter quantification.

Quality assurance will be maintained through procedures documented in this QAPP and reported semiannually to EPA.

Figure 3: Water Quality Monitoring Sites



Quality Objectives for Measurement Data (A7)

This project will benefit homeowners and citizens in the Ponchitolowa Watershed by improving water quality in residential neighborhoods served by ATUs- many of which are demographically classified as LMI. Observing and recording the behavior of the actual system through the data collected as described in this QA/QC plan will accomplish this purpose. The improved water quality will reduce the risk of water-borne pathogens and mosquito-borne disease vectors. Citizens will also benefit from education on wastewater and water quality issues. Finally, municipalities will benefit from the tools and strategies report developed through this project.

A representative data sheet to be used for field data collection is included as *Figure 4*. All sample collection, sample handling, analytical methods, and *in situ* monitoring methods to be performed in the water quality sampling regime will be in accordance with the *Standard Methods for the Examination of Water and Wastewater, 23nd Ed* (2017). Tulane University will conduct the analytical analyses required in this project in accordance with their quality management program.

Data will be stored in a Microsoft Excel database. It will be subjected to quality control and descriptive statistics as described in Guidance for Data Quality Assessment (EPA QA/G-9) using Microsoft Excel and SAS, SPSS, or similar statistical program.

- Semiannual reports will be submitted on the following dates each year: March 31st and September 30st.
- Statistical analysis and relative percent difference (RPD) will be computed for each parameter. The data will be included in the final report.
- The Analysis of Variance (or non-parametric equivalent- Kruskal-Wallis analysis) and/or categorical data analysis will be employed to evaluate the statistical relationships between parameters. Probability of $\alpha \leq 0.05$ is considered of statistical significance with a null hypothesis stating no significant difference between parameters between baseline and post inspection sampling.
- Linear regression and moving averages will be used to assess change over time for individual parameters.
- Spearman's Rho will be utilized to assess correlative relationships between tested parameters vs. system repairs, once again with the null hypothesis of no difference before or after.
- Summaries of the analyzed data and QA procedures will be presented in reports.
- The ultimate goal of the statistical analysis is to assess the relationships between the various parameters and determine if there are statistically significant reductions in any parameters between our background sampling and end of project sampling, as well as over the course of the project, as they relate to 303(d) listing.
- The extensive list of parameters that will be monitored/sampled in the study are provided in *Table 2* of the QAPP.
- It is also a goal of the program to have the project and results published in a peer reviewed scientific/environmental journal.

Special Training Requirements (A8)

The water monitoring personnel conducting the analysis have been trained in the collection of the water samples. The LPBF Water Quality Field Coordinator will conduct other general procedure training. All staff collecting water quality samples have also completed EPA's Quality Assurance Training. While no specific certification for water quality field data collection is required, LPBF will follow the *Policy to Assure the Competency of Organizations Generating Environmental Measurement Data Under Agency-Funded Assistance Agreements* (EPA, 2013).

Documentation and Records (A9)

All associated EPA personnel, LPBF project personnel, and the contracted lab will receive copies of this QAPP and subsequent updates/revisions. The QA Technical Manager is responsible for ensuring all parties have the most up-to-date project QAPP. Water monitoring personnel will receive copies of the sampling standard operating procedure with all standard methods employed explained in full detail. Records maintained include the following: all data relating to sampling, analysis, and quality control; documentation on equipment upkeep and calibrations for preventative maintenance; documentation of errors and corrective actions; and all performance evaluations. Semiannual reports will be generated to assess progress of the project and address any technical difficulties, which may necessitate a change of the original project design. These Semiannual Reports will be submitted to EPA for review and approval.

Documentation and records pertaining to the project will be maintained by LPBF electronically for at least 7 years after the close-out of the project. Backups of LPBF's entire shared network are kept both onsite (daily backup) and offsite (biweekly backup). When a file is archived, it is moved to a shared archive folder on the network, which is backed up offsite every two weeks. Archives are maintained indefinitely until files are no longer needed.

Figure 4: Data Sheet

Water Quality Monitoring- Data Form

Monitors: ______________________________

Date: ______________ **Time:** ______________

Recent Weather Conditions: ______________________________

____

Site, Time, Turbidity	Water Temp	Dissolved Oxygen	Specific Conductance	pH
Site:				
Time:				
Turbidity:				
Mean				
Site:				
Time:				
Turbidity:				
Mean				
Site:				
Time:				
Turbidity:				
Mean				
Site:				
Time:				
Turbidity:				
Mean				
Site:				
Time:				
Turbidity:				
Mean				

Site, Time, Turbid	Water Temp	Dissolved O_2	Spec Cond	pH
Site:				
Time:				
Turbidity:				
Mean				
Site:				
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Site:				
Time:				
Turbidity:				
Mean				

Calibration/Maintenance- Initial completed tasks **Date:** ______________

__________ - **D.O. Tip Changed**
__________ - **Conductivity/Salinity Checked Against Distilled Water**
__________ - **pH Solutions Changed** (7 and 10 buffers)
__________ - **Turbidimeter checked with secondary standards**
Readings marked on standards: ______ ______ ______
Readings taken from turbidimeter: ______ ______ ______

Sampling Process Design (B1)

The experimental design and monitoring activities have been discussed in section A6. Sampling will begin with the approval of the QAPP. All project personnel have completed training. This project period will last one year to ensure that an adequate sample size will be attained for analyses.

- For each PC site, physiochemical, nutrient, organics, and bacteriological parameter analyses will be performed three (3) times as a baseline then monthly for ten (10) months, totaling thirteen (13) measurements annually.

All parameter measurements will be analyzed according to *Standard Methods for the Examination of Water and Wastewater, 23rd Edition* (2017).

Sampling Methods Requirements (B2)

Physiochemical Parameters

Dissolved Oxygen, Water Temperature, Specific Conductance, pH, and Turbidity will be measured *in situ* by meters, as outlined in *Section B4*. All instrumentation measurements (e.g. "meter" measurements) will be taken immediately beneath the surface, as some sites are shallow. At each site, three measurements will be taken and averaged for each parameter as the daily value except for turbidity measured by the turbidimeter. For the turbidimeter the average function is utilized to average 10 readings. The LPBF Water Quality Program Director will be responsible for coordination of analyses and corrective action, if necessary. Water quality sampling Standard Operating Procedures (SOPs) are maintained at the following web address: https://saveourlake.org/wqsop/

Microbiological, Analyses

Sampling methods requirements for all parameters collected for lab analysis are described in Table 1 below. Information in Table 1 is from *Table 1060:I. Summary of Special Sampling and Handling Requirements* (Standard Methods, 2017).

Table 1: Sample Collection and Holding

Parameter	Type of Sample	Sample Size	Holding Time	Holding Conditions	Other Instructions
Fecal Coliform	grab	100 mL	6 hours	Cool, ≤6°	Add thiosulfate or other quenching agent if Chlorine is present
E.coli	grab	100 mL	6 hours	Cool, ≤6°	Add thiosulfate or other quenching agent if Chlorine is present

Samples will be collected in accordance with *Standard Methods for the Examination of Water and Wastewater* Methods 1060B and 9060A:

- Bacteriological samples will be collected in 120 ml vessels (sealed, sterilized, plastic water sampling bottles).
- In the case of non-sterile procedure or other sampling procedure failure, the collection bottle will be discarded and another will be labeled and employed.

- All samples will be stored in a cooled ice chest (< 8 ºC, SM 9060 B) and transported to the lab within six hours of collection, in accordance with *Standard Methods for the Examination of Water and Wastewater* Methods 1060C and 9060B.
- Upon receipt of samples in the lab, the temperature of the samples is checked, and all samples will either be processed immediately or placed in a refrigerator (< 8 ºC), not to exceed two hours before processing.

Pathogen Analysis by DNA/RNA

Pathogens can be detected by counting nucleic acid sequences deoxyribose nucleic acid (DNA) and Ribose Nucleic Acid (RNA). These methods enumerate difficult to culture microbes by counting the genetic targets that are specific to the pathogen of interest. This method utilizes the primers and genetic machinery associated with gene expression. Quantitative PCR is used by microbiologists working in the fields of food safety, food spoilage and fermentation, environmental sciences, and for the microbial risk assessment of water quality and in public health protection.

qPCR may also be used to amplify taxonomic or functional markers of genes in DNA taken from environmental samples.[34] Markers are represented by genetic fragments of DNA or complementary DNA. By amplifying specific-target genetic elements, researchers can quantify the amount of the element in the sample prior to amplification. Using taxonomic markers (ribosomal genes) and qPCR can help determine the amount of microorganisms in a sample, and can identify different families, genera, or species based on the specificity of the marker.

While these procedures are useful in building understanding of pathogens in the environment, they are not yet codified as accepted NPDES analytical procedures in *40 CFR 136 Table IA*. The methods employed for this testing and the interpretation of the results **are not intended to be used for regulatory purposes**.

LPBF will be responsible for the collection of samples as identified in this QAPP, and will relay samples to the Tulane University (TU) laboratory[3].

Detection of Microbial Pathogens by PCR

TU will be charged with the detection of selected waterborne pathogens using the electronegative filter method: Briefly, 2.5 M $MgCl_2$ will be added to collected 1L wastewater samples to obtain a final concentration of 25 mM. Samples will be subsequently passed through an electronegative filter with a 0.45 µm pore size (cat. No. HAWP-090-00; Millipore, Billerica, MA) attached to a glass filter holder (Advantec, Tokyo, Japan). Magnesium ions will be removed by passing 200 mL of 0.5 mM H_2SO_4 (pH 3.0) through the filter, and the viruses will be eluted with 10 mL of 1.0 mM NaOH (pH 10.8). The eluate will be recovered in a tube containing 50 µL of 100 mM H_2SO_4 (pH 1.0) and 100 µL of 100x Tris-EDTA buffer (pH 8.0) for neutralization. Further centrifugation will be performed using a Centriprep YM-50 containing a membrane with a nominal molecular weight limit (NMWL) of 50 kDa (cat. No. 4310; Millipore) to obtain a final volume of approximately 650 µL. Concentrates will be processed for immediate extraction of viral nucleic acids.

[3] The principle of the HA negatively charged membrane method as previously described (Katayama et al., 2002) with slight modification.

The filters will be then used to extract bacterial DNA, and the extracted DNA will be used for the detection of these bacterial pathogens*: E. coli 0157, Campylobacter, Enterotoxigenic E. coli, E. coli STEC, Salmonella, Shigella, Vibrio cholerae*.

Viral DNA and RNA will be extracted from the virus concentrates spiked with Murine Norovirus (MNV) process control using the ZR Viral DNA/RNA Kit (Zymo Research, Irvine, CA) to obtain a final volume of 100 µL, according to the manufacturer's protocol. The Reverse Transcription (RT) reaction will be performed using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA). Briefly, 10 µL of extracted RNA will be added to 10 µL of RT mixture containing 2 µL of 10x reverse transcription buffer, 0.8 µL of 25x deoxynucleoside triphosphates (dNTPs), 2 µL of 10x random hexamers, 50 units of Multiscribe™ reverse transcriptase, and 20 units of RNase inhibitor. The RT reaction mixture will be incubated at 25°C for 10 min, followed by 37 °C for 120 min, and a final 85°C for 5 min to inactivate the enzyme.

Quantification of viral pathogens by PCR

Taqman-based qPCR assays for selected viruses (Norovirus, Rotavirus, Adenovirus) will be performed with the iQ5 Real-time PCR Detection System (Bio-Rad Laboratories). Reaction mixtures (25 µL) consist of 12.5 µL of iQ Supermix (Bio-Rad Laboratories, Hercules, CA), forward and reverse primers, probe(s), and 2.5 µL of (c) DNA template. Serial tenfold dilution of the standard plasmid DNA containing inserts of the amplification region or gblock will be used to generate a standard curve. All qPCR reactions will be performed in duplicates, and positive as well as negative controls will be included in the qPCR reaction plates to avoid false-positive and negative results.

Detection of Mosquitos and Mosquito Fish Populations

Fish presence will be measured by a thirty-second visual inspection of the water surface in a one square meter area around the septic outfall pipe. If no outfall pipe is present, the one square meter observation area will be the nearest culvert opening. Presence-absence monitoring is a common measurement in fish ecology (Manel et al. 2001), and visual observations as population estimates are a standard referenced in *Biological Examination: Fishes*. Considering the shallow water present in ditches and the ease with which fish can be visually observed, we believe a set amount of time spent visually monitoring for fishes to be analogous to underwater observations.

In the same one-square meter block, water will be collected by mosquito dipper for water quality measurements from directly in front of the outlet pipe. If no or insufficient water is present under the pipe, water will be retrieved from the closest location to the outlet pipe containing sufficient water for probing. Most septic outfall ditches do not contain enough water to perform *in situ* measurements of water quality. Therefore, collected water will be held in a receptacle for measurement.

Following water quality measurements, a mosquito dipper will be used to measure a standardized sample of mosquitoes (*Mosquito Ecology,* Silver 2007) up to 5 times in the same one-meter block until mosquito egg rafts/larvae/pupae are found. Additional data including presence of the outfall pipe, flowing water, and a series of digital images will be collected at each site.

Sample Handling (B3)

Physiochemical Parameters

All physiochemical measurements are to be performed *in situ.* Data will be recorded on the field data form (*Figure 4*).

Microbiological Analyses

Sample handling procedures for microbiological, nutrient, and organics analyses are presented in Section B2. Samples will be collected by the water monitoring personnel, delivered by him/her to the lab, and personally handed to the appropriate lab personnel. Sample labeling, handling, and disposal within the lab will proceed in accordance with their quality assurance manual (QAM).

Analytical Methods Requirements (B4)

Physiochemical Parameters

The analytical methods to be employed for this study are summarized in this section (Table 2).

Table 2: Standard Methods for Parameters Tested

Parameter	Method	Equipment
Dissolved Oxygen	Standard Methods for Examination of Water and Wastewater, 23rd Ed. Method 4500-OG	YSI Pro 2030 Meter 0-20mg/L range, ± 0.2mg/L accuracy
Temperature	Standard Methods for Examination of Water and Wastewater, 23rd Ed. Method 2550 B	YSI Pro 2030 Meter -5 to +55°C range, ± 0.3°C accuracy
Specific Conductance	Standard Methods for Examination of Water and Wastewater, 23rd Ed. Method 2510 B	YSI Pro 2030 Meter 0 to 200 mS/cm range, ± 0.1 mS/cm accuracy
Turbidity	Standard Methods for Examination of Water and Wastewater, 23rd Ed. Method 2130 B	Hach Portable Turbidimeter 0 to 1000 NTU range, ± 0.01 NTU accuracy
pH	Standard Methods for Examination of Water and Wastewater, 23rd Ed. Method 4500-H^+B	YSI pH 10A pH/ Temperature Pen 0 to 14.00 range, ± 0.02 pH ± 1 LSD accuracy

Microbiological Analysis

Fecal coliform and *Escherichia coli* are the bacteria examined as indicators for the enteric pathogen level. Fecal coliform is utilized as it is the Louisiana standard and for the continuation of data from previous LDEQ analysis at Ambient monitoring stations.

- The lab will utilize *Colilert 18* and *Colilert 18 Alternate Test Methods* for enumeration of fecal coliforms and *E. coli*, as detailed in 40 CFR 136.3 table IA. For this method, the detection limit is MPN 1/100ml.

Quality Control Requirements (B5)

The quality control performed on a set of samples is dictated by the protocols of the individual methods. All quality control methodology and statistics are performed in accordance with Methods 1020B&C, 1030A, the parameters' test methods in *Standard Methods for the Examination for Water and Wastewater,* the manufacturers' guides, and the *Guidance for Data Quality Assessment* (EPA QA/G-9). For general data error control, triplicate sampling is employed for physiochemical parameters to produce a mean and relative percent difference value for each parameter at each site.

The lab will perform all of its quality control requirements in accordance with its QAM.

Field Replicates
Bi-annually a replicate water sample will be collected (sequentially, at the same location after sample collection) at one of the sampling sites and submitted to the laboratory as a blind sample. The replicate data will be utilized and analyzed as quality control values. In addition, all meter measurements are taken in triplicate at all sampling sites for QA purposes.

Field and Laboratory Blanks
Bi-annually one blank for fecal coliform will be collected by pouring distilled water into the collection bottle in the field and submitting it to the lab with the other samples. The QC goal is no growth in the sample, which would appear as <1.8 MPN on the data form. Laboratory blanks will be run under the lab's QA plan.

Matrix Spikes/ Spike Duplicates
Matrix Spikes/ Spike Duplicates are not necessary for the analysis of physiochemical parameters, as all tests are conducted *in situ*. Matrix Spikes and Spike Duplicates associated with the collection and analysis for nutrients, organics, and bacteriological parameters are detailed in lab's QAM.

Analysis of Quality Control Data
Quality control data will be summarized in the final report and utilized to assess the overall precision, accuracy, and completeness of each method. For these methods, the precision and accuracy is assumed to approximate published values.

Assessing Data Precision, Accuracy, and Completeness

1) Precision

On each sampling date, three readings for each physiochemical parameter will be taken at each site. The triplicate data will be subjected to precision analysis. Precision will be expressed as the relative percent difference (RPD). Microsoft Excel will be used for these calculations.

$$RPD = (X^1-X^2) / (X^1+X^2/2) * (100)$$

Where X^1 and X^2 are maximum and minimum sample values from daily triplicate samples

2) Accuracy

Accuracy is a measure of the closeness an experimentally observed value and the actual value, the latter of which is determined by the analyst through the use of sample spikes, surrogates, or reference standards. Field meters will be considered to be giving accurate readings through calibration with NIST standards and equipment maintenance (as per correspondence with EPA technical rep.) See Calibration and Maintenance schedule (Table 2) below for upkeep activities.

3) Completeness

Completeness is the amount of valid data generated in relation to the total amount of data produced for a given analytical method. Valid data is defined as data with associate QA/QC measurements that fall within required values for the purpose of this study (Table 2). Data completeness goals for each parameter are also noted in Table 3.

Evaluation of Statistically Derived QA/QC Data

Data that has been generated for QA/QC purposes must be assessed to determine the ability of the equipment and personnel to generate reliable data. Microsoft Excel will be used for these calculations. The data completeness results will be notated in the final report for the program. Completeness goals are noted in Table 3 for each parameter.

Table 3: Criteria for QA/QC Field Parameters

Parameter	Relative % Difference	Standard Method	Completeness Goal
Dissolved Oxygen	10	Ref1/4500-OG	> 90% data/ year
Specific Conductance	5	Ref1/2510B	> 90% data/ year
Turbidity	10	Ref1/2130B	> 90% data/ year
Temperature	5	Ref1/2550B	> 90% data/ year
pH	5	Ref1/4500-H^+B	> 90% data/ year

Microbiological Analyses

All quality control requirements for the microbiological portion of this research will be conducted by the lab in accordance with their QAM.

Instrument/Equipment Testing, Inspection, and Maintenance Requirements (B6)

Physiochemical Parameters

All equipment and associated components will be inspected, calibrated, and tested by the Water Quality Field Coordinator upon receipt according to the operator's manual. Equipment will be maintained according to the operator's manuals. In the case of equipment failure, the piece of equipment will be sent to a reputable company for repairs. Equipment will be inspected, calibrated, and tested by the Water Quality Field Coordinator upon receipt. Back-ups for all equipment and spare parts are maintained by the LPBF. Water monitoring personnel perform routine maintenance on all equipment in accordance with the equipment operator's manuals.

Microbiological, Nutrient, and Organics Analyses

The lab tests, inspects, and maintains its own equipment in accordance with its QAM.

Instrument Calibration and Frequency (B7)

Physiochemical Parameters

Calibration protocols will be performed under the following conditions:

1) First use of an analytical instrument, component of the analytical instrument, or analytical method;
2) During the sample analysis procedure, as dictated by the methodology;
3) After instrument repair and/or maintenance;
4) After quality control check failure.

Additional calibration requirement and procedures recommended by the instrument manufacturers' will also be followed. All calibrations will be performed according to the operator's manual using standard solutions purchased from reputable suppliers (standardized against NIST-certified references). All calibrations will be performed in accordance with the procedures specified in the analytical methodology commanding their use (Table 4).

Table 4: Physiochemical Instruments Calibration/Maintenance Procedures

Equipment	Schedule	Procedure
Dissolved Oxygen Probe	Each Use/Weekly→ Monthly→ Bi-Annual→	- Calibrate to distilled water - Change tip and replace solution - Check against standard chart, - Clean anode & cathode
Salinity/Conductivity Meter	Bi-Annual/ Repair→ Monthly→	- Calibrate to one standard KCl solution - Check salinity against distilled water (0 ppt salinity)
Turbidity	Quarterly→ Monthly→	- Calibrate to formazin standard - Check against secondary standards
Thermometer	Bi-Annual→	- Check against similar probe
pH meter	Each Use/Weekly→ Monthly→	- Perform two point calibration - Change all buffers and solutions

Microbiological, Nutrient, and Organics Analyses
The lab standardizes and calibrates all of its equipment in accordance with its QAM.

Inspections/Acceptance Requirements for Supplies and Consumables (B8)

All equipment and supplies are purchased from reputable dealers. The Water Quality Field Coordinator logs the receipt of all new equipment and inspects, calibrates, and tests the equipment (as necessary) before accepting them. If equipment/supplies are damaged or do not pass calibration and testing, they will not be accepted. All supplies are handled and stored according to operator's instructions.

Microbiological, Nutrient, and Organics Analyses
During sample collection, the monitoring personnel will be responsible for inspection and acceptance of the sample containers. The lab will inspect its own consumables and supplies in accordance with their QAM.

Non-Direct Measures (B9)

There are no non-direct measures used in this research.

Data Management (B10)

Data management follows the chart below, in *Figure 5*. The results of all monitoring and analyses will be put into a database by the LPBF monitoring personnel and verified by the Water Quality Field Coordinator. Researchers requesting access to data will obtain access upon signing of the data-sharing agreement by the requesting investigator and an authorized representative of the recipient institution. Upon receipt, data will be provided in password-protected files. LPBF has implemented data management protocols to "block data" or report data in a neighborhood or area level, not as the individual or direct postal addresses. By reporting neighborhood or area-level of pathogen presence and failure rates, LPBF looks to mitigate identifying individuals or families with personally identifiable information. Data will also be made available to national repositories based on the type of data (e.g., EPA WQX, NOAA, PubMed, etc.) following publication.

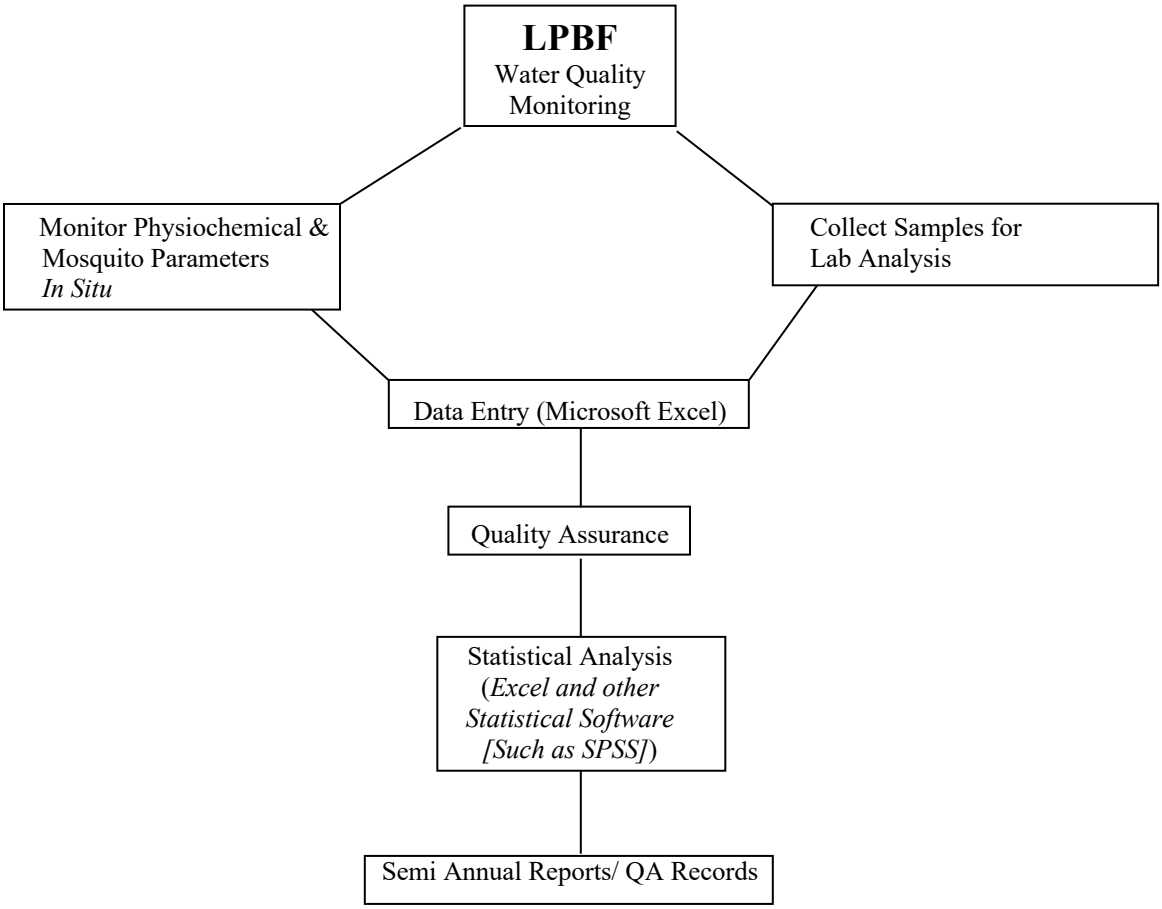
Microsoft Excel or JMP, a SAS program, or similar statistical program will be utilized for statistical analysis.

All activities will be performed by LPBF, TU, or STPMAD, as reflected in the shared data agreement. A formal request delineating the data-sharing agreement will include the following in addition to the guidelines described above:

1) a commitment to using the data only for research and practice purposes and not to identify any individual participant,
2) assurance that data will be used in accordance with Federal (e.g., NIH, NOAA) Public Access Policy regarding submission to peer-reviewed journals and submission of the published manuscript to the appropriate digital archives no later than 12 months after publication;
3) assurance that the original values within the dataset will not be altered in any way;

4) assurance that the data will be secure, using appropriate computer technology, and that the data will not be distributed to a third party;
5) assurance that the data will not be used for commercial purposes or to raise money by an individual or affiliated organization; and
6) acknowledgment that the EPA grant is the data resource in scientific publications.

Figure 5: Data Management Flow Chart



Assessment and Response Actions (C1)

Assessment activities needed for this project include performance evaluations, performance reviews, and peer review. The LPBF Water Quality Program Director (Principle Investigator) and LPBF Water Quality Field Coordinator will be in constant contact with the LPBF monitoring staff to resolve issues as they arise. In addition, all data will be subject to QA review, which will be summarized in the final report. The lab will conduct its own assessment of its methodology in accordance with its QAM.

Performance Evaluation

Monitoring personnel are evaluated for their knowledge and ability to carry out the required measurements. The monitor for this project has already been trained in water monitoring and sample collection methodology. Performance evaluations will be completed before project personnel are allowed to participate and repeated an additional time during the project. The Water Quality Field Coordinator and/or Principle Investigator will view the data monthly and upon receipt. If any issues arise, the Principle Investigator will work with the monitor to resolve the issues.

Performance Reviews

Performance reviews will be conducted to document the taking of measurements and the treatment of data from time of collection to final reporting of results at least once per year. The primary goal of the review will be to detect deviations from the standard operating procedures and to make corrective adjustments. The Principal Investigator will be responsible for implementing corrective procedures and monitoring the progress of the personnel.

Peer Review

Data quality will be evaluated by peer review and information exchange and consultation with other research parties involved with similar projects. Publication of project results in peer reviewed scientific journal is desired.

Reports to Management (C2)

The following project reports will be prepared by LPBF and submitted to the EPA Project Officer.

Progress Reports

Progress reports will be prepared by LPBF in accordance with grant work plans. Semiannual project reports will share all data collected and analyzed to date, address any problems that may affect the quality of the data (including corrective actions performed), and be an evaluation of the status of the project. Semi-annual and final reports will summarize, analyze, and graph all project data to draw conclusions on the project and report all QA/QC findings.

Data Review, Verification, and Validation (D1)

A comprehensive review and verification of quality assurance elements will be conducted after data collection is complete, including: assessment of data entry, transcription, and calculation errors; use of acceptable sampling methods; verification that holding times for those parameters analyzed by a lab were met; meters were properly calibrated for each use; use of correct containers and preservatives; verification that field blanks and duplicates were collected as planned and that they met aforementioned QC acceptance criteria; verification that the number of samples planned for collection were collected as planned; verification that sites listed for sampling were actually sampled; and verification that completeness goals were met for each parameter. Any departures from these types of project planning criteria listed in the QAPP will be noted in project reports.

Verification and Validation Methods (D2)

Section A7 discusses the responsibilities of LPBF in this study, and Section B10 discusses the chain of custody for all accumulated data. The water monitoring personnel are responsible for verifying the completeness and correctness of the data through the custody and transferal process. The LPBF will perform the quality assurance and validation analysis to assure that the data complies with QA/QC criteria and that all instruments comply with operational standards.

The following data verification methods will be employed:

- At least 10% of field data sheets will be randomly compared with the database to verify correct data transcription.
- Sample delivery sheets will be checked to verify holding times and preservation requirements for microbiological samples.
- Calibration logs will be consulted to verify that meters were properly calibrated.
- Duplicates will be verified against precision targets listed in Table 2.
- Blanks will be verified against assessment criteria for field blanks described in Section B5.
- Number of samples collected will be compared to the total number originally planned for each parameter.
- Completeness (defined in Section A7) will be assessed using the following equation: Completeness = (Total valid samples / Total samples collected) (100)
- Field data sheets will be reviewed to verify sampling locations were sampled as planned.
- Experimental controls that are not within limits and/or when duplicate samples vary significantly, the data will be rejected. Data acceptance will be the responsibility of the Principal Investigator.

Reconciliation with Data Quality Objectives (D3)

LPBF will reconcile the data with the quality assurance process outlined in the LPBF QMP. LPBF will verify that pertinent data results are acceptable by intercomparison checks, performance evaluations, and evaluations as described previously in Section C1. Data that does not meet the data quality requirements will be rejected after review by the Principal Investigator.

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